"Effects of Trichothecenes on Cardiac Cell Electrical Function"

ANNUAL REPORT

W.T. Woods, Jr.

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This year was devoted to elucidating the effects, if any, of intravenous T-2, roridin A, and other trichothecenes on the mammalian cardiovascular system. Effects were studied at whole animal and single cell levels. Reflex, cardiac, and hemodyramic effects were assessed in whole animals while specific electrophysiologic effects on single cell action potentials were observed in isolated, perfused cardiac tissue where microelectrodes could be used and where neurohumoral factors could be controlled. These effects are described in detail in the Experimental Results section. Briefly, certain trichothecenes were observed to have direct effects on the heart and also on the peripheral circulation. However, there were frequently simultaneous effects on autonomic neural control (i.e. reflexes) of the cardiovascular system and further work must be done to unravel these complex interactions.

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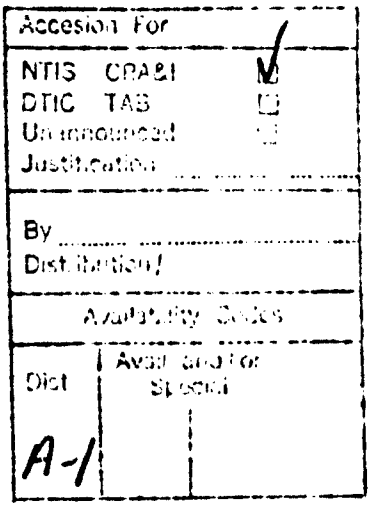
FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-123, Revised 1978).



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General aims of the research from 1 October, 1983 through 30 September, 1984.

This year was devoted to elucidating the effects, if any, of intravenous T-2, roridin A, and other trichothecenes on the mammalian cardiovascular system. Effects were studied at whole animal and single cell levels. Reflex, cardiac, and hemodynamic effects were assessed in whole animals while specific electrophysiologic effects on single cell action potentials were observed in isolated, perfused cardiac tissue where microelectrodes could be used and where neurohumoral factors could be controlled. These effects are described in detail in the Experimental Results section. Briefly, certain trichothecenes were observed to have direct effects on the heart and also on the peripheral circulation. However, there were frequently simultaneous effects on autonomic neural control (i.e. reflexes) of the cardiovascular system and further work must be done to unravel these complex interactions.

EXPERIMENTAL RESULTS

EFFECTS OF INTRAVENOUS T-2 AND RORIDIN A ON THE CANINE CARDIOVASCULAR SYSTEM. (Woods and Bubien)

Progress

Animals weighing 20 ± 5 kg. were anesthetized with intravenous pentobarbital (30 mg/kg). T-2 toxin or roridin A (0.1, 1.0, and 3.0 mg/kg) were injected in one intravenous bolus of dimethyl sulfoxide (DMSO). Each injection was preceded by an equivalent volume of toxin-free DMSO to serve as a control for effects of DMSO per se. In 5 experiments certain responses to these toxins were immediate, but some required up to 2 hr. to develop. There was always a transient fall in arterial pressure and increase in heart rate. When this injection included T-2 toxin, there was after 5 min. a progressive increase in heart rate that reached a stable peak after 60 ± 15 min. (Figure

1B). In 4 separate experiments, for example, the increase was from 145 ± 6 to 195 ± 10 beats per min. (sinus tachycardia). During the period of increasing heart rate, arterial pressure was not significantly lower. This suggests that the elevated heart rate might not be a reflex-mediated response (to hypotension, for example). However, experiments were performed to test the role of norepinephrine which is the main sympathetic neurotransmitter in the mammalian heart. Propranolol (250 micrograms/kg.) was injected intravenously during T-2-induced tachycardia to block the beta-adrenergic receptor activated by norepinephrine (Figure 1C). In 3 experiments, this lowered heart rate, but only eliminated 1/2 of the T-2-induced increment in heart rate. Therefore, the data suggest that effects of T-2 on heart rate are mediated by neural release of norepinephrine as well as a direct effect on pacemaker cells.

The same number of experiments were performed in the same way to assess the cardiovascular effects of roridin A. Responses were identical to those observed after intravenous T-2 except that 75 ± 30 min. after roridin A the heart rate suddenly fell to a level suggesting sinus arrest or sino-atrial block of conduction (Figure 2). Electrocardiograms suggested that sinus arrest with emergence of a substitute pacemaker had taken place. Another marked response was the increased T-wave amplitude (Figure 3).

Plans

1. Investigate the mechanisms by which T-2 toxin and roridin A elicit tachycardia and, subsequently, pacemaker arrest.
2. Determine whether the autonomic nervous system plays a major role in T-2 and roridin A toxicity.
3. Determine whether T-2 and roridin A have effects attributable to blockade of the slow channel.

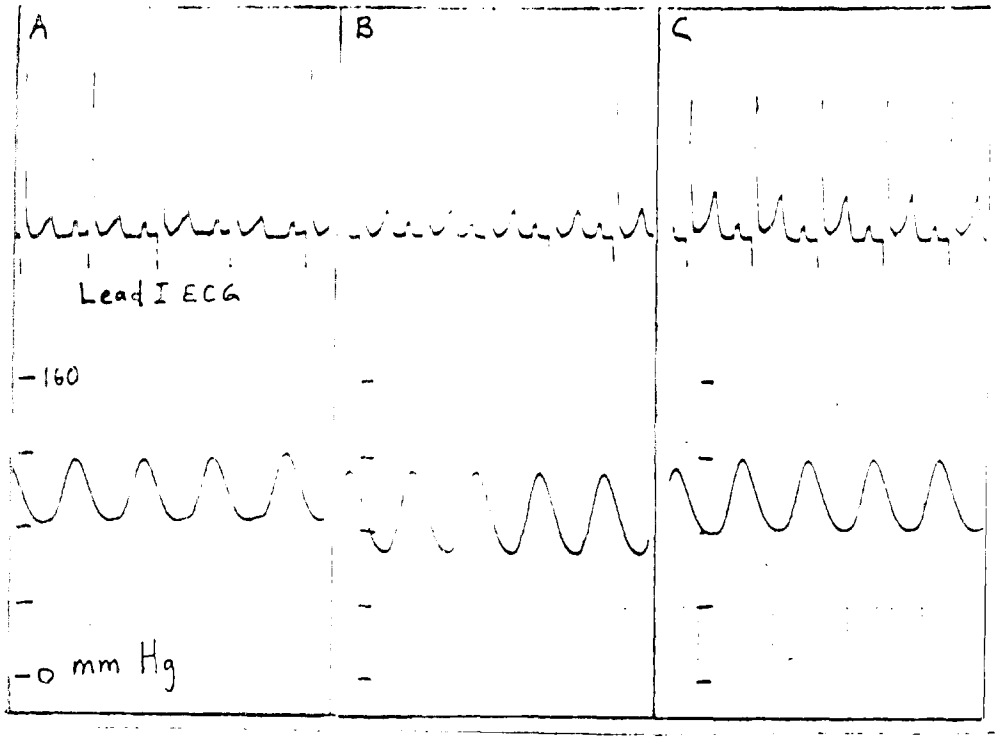

Figure These panels show a lead I ECG (upper) and arterial pressure (lower) in an anesthetized animal before intravenous T-2 (1.5 mg./kg., panel A), 2 hours after T-2 (panel B), and 1 hour later following injection of propranolol (5 mg.) (panel C) 1.0 cm = 0. 400 sec. Note especially that only part of the T-2-induced tachycardia (150 to 176 bpm) was blocked by propranol (165 bpm). There was a time-dependent increase in T-wave amplitude suggesting hyperkalemia, but P-waves remained prominent suggesting the opposite.

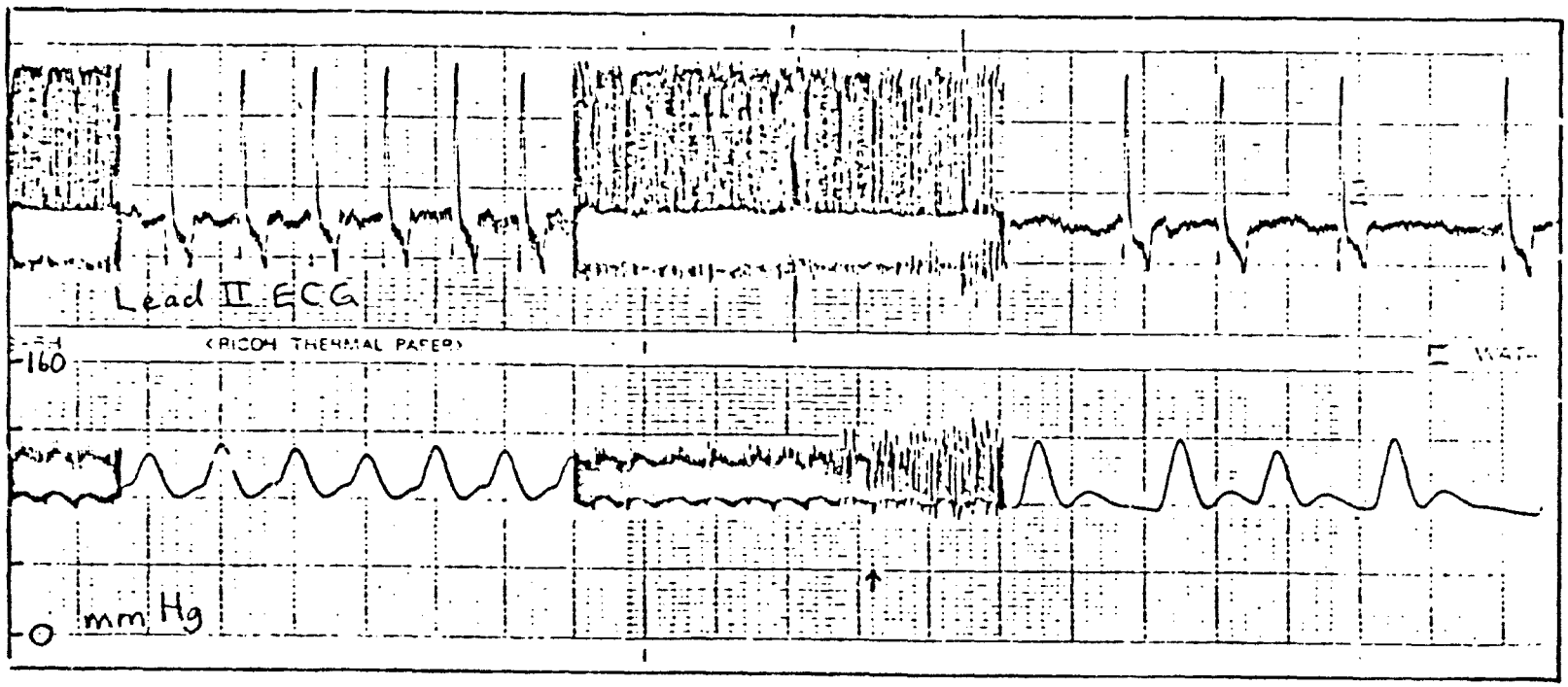

Figure 2. This continuous record shows the transition from normal impulse conduction to second degree atrioventricular block observed after 1 hour of intravenous roridin A 2.0 mg./kg. The lead II ECG (upper trace) and arterial pressure trace (lower trace) show the irregular rate associated with this arrhythmia which began approximately at the arrow. Fast speed 25 mm./sec. and slow speed = 25 mm./min.

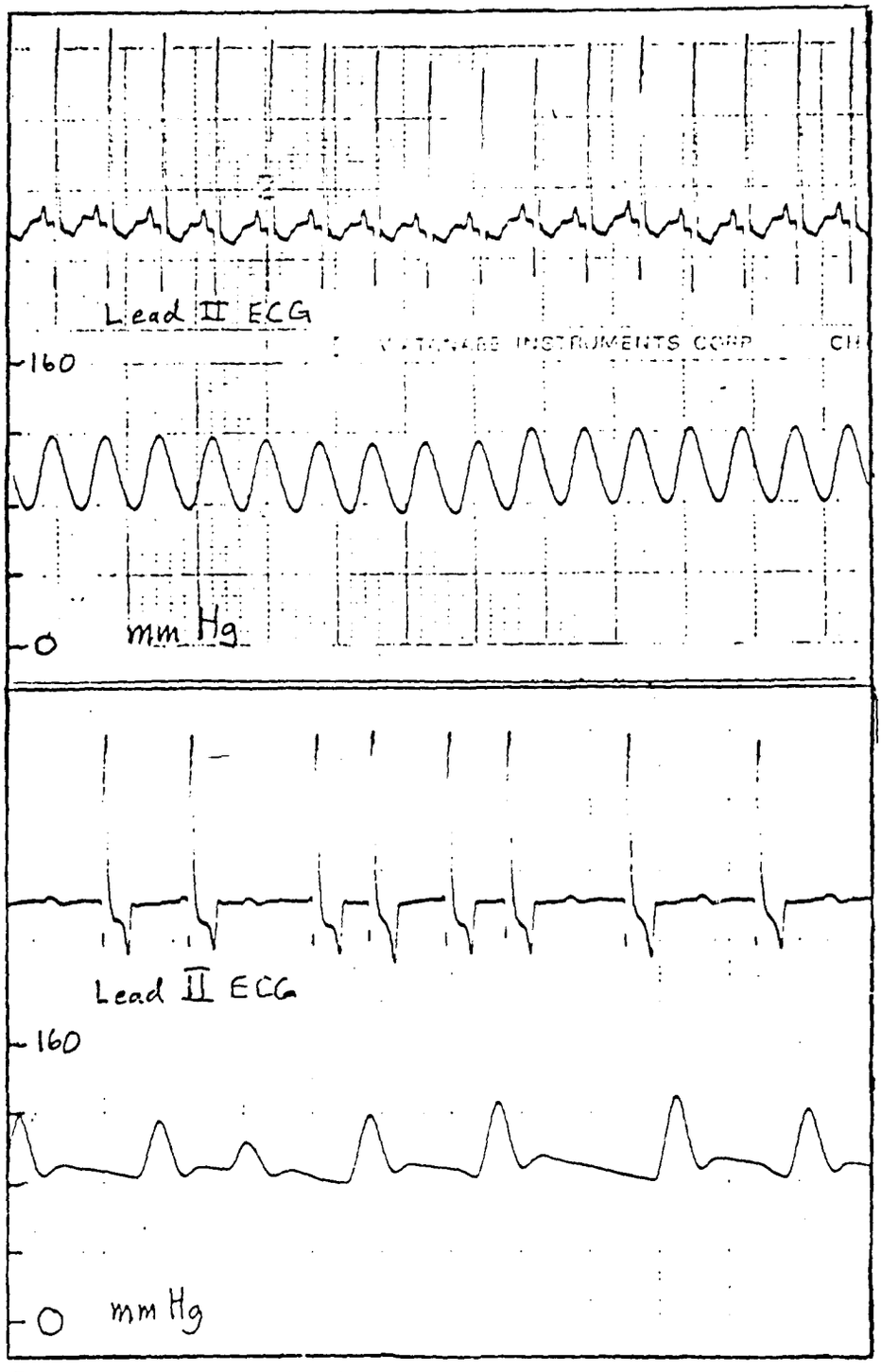

Figure 3. These two records (from the same experiment as Figure 2) show how roridin A prolonged the PR interval (80 msec. to 320 msec.) and markedly increased T wave amplitude (negative in the canine lead II ECG). Upper panel was before, and lower panel was 2 hr. after roridin A was injected.

Table 3 summarizes the effects of scirpentriol on the action potentials of canine false tendon cells, papillary muscle cells, and ventricular muscle cells. The R2, R3 hydroxylated metabolite had no significant effect on the action potential parameters of canine false tendon cells or papillary muscle cells. Ventricular muscle cell action potentials however were significantly altered by scirpentriol. The action potential duration was shortened ($p < 0.05$), the cells were depolarized by 11.5 mv ($p < 0.05$), and the total amplitude was reduced by approximately the same amount ($p < 0.05$). T-2 altered these parameters in the false tendon cell action potentials, and had no effect on the ventricular muscle cells. Scirpentriol had no effect on the false tendon cells but significantly altered ventricular muscle cell action potentials.

Table 4 summarizes the effects of T-2 tetraol on canine false tendon cell, papillary muscle cell and ventricular muscle action potentials. The hydroxylated metabolite had no effect on the false tendon cell action potential parameters. T-2 tetraol depolarized papillary muscle cells by 16.5 mv ($p < 0.05$), which was reflected in the reduction of the total amplitude ($p < 0.05$), and also reduced dV/dTmax by 50% ($p < 0.05$). In ventricular muscle T-2 tetraol reduced the action potential duration ($p < 0.05$), but no other parameters were altered.

Table 5 shows that T-2 shortened the action potential duration in papillary muscle cells ($p < 0.05$), and similarly, scirpentriol shortened the action potential duration of ventricular muscle cells ($p < 0.05$). The addition of adenosine to the suffusate had no effect on the shortened action potentials. ATP (2 mM/L) produced no changes in action potential parameters of papillary muscle cells or ventricular muscle cells from the controls. However ATP counteracted the effect of T-2 on the papillary muscle cell action potential duration and it also counteracted the shortening effect of scirpentriol on the ventricular muscle cell action potential durations.

Table 1. Control action potential parameters for 3 ventricular cell types

	AMPLITUDE (mv)	OVERSHOOT (mv)	dV/dT max. (V/S)	COND. VEL. (M/S)	MDP (mv)	APD (20) (ms)	APD (50) (ms)	APD (8 (ms)
FALSE TENDON								
X	119.9	31.2	258.5	0.953	87.8	40.8	150.8	208.5
sd	9.2	5.5	45.3	0.325	8.1	8.6	37.5	34.3
(n)	(13)	(13)	(13)	(10)	(13)	(13)	(13)	(13)
PAPILLARY MUSCLE								
X	98.1	22.9	151.3	0.190	75.5	105.3	190.3	230.0
sd	8.6	3.1	39.5	0.040	10.2	33.4	46.1	55.8
(n)	(15)	(15)	(12)	(5)	(15)	(15)	(15)	(15)
VENTRICULAR MUSCLE								
X	98.2	21.0	127.1	0.178	77.5	107.1	206.5	246.2
sd	10.3	4.4	35.8	0.126	8.0	23.6	25.3	26.0
(n)	(17)	(17)	(14)	(8)	(17)	(17)	(17)	(17)

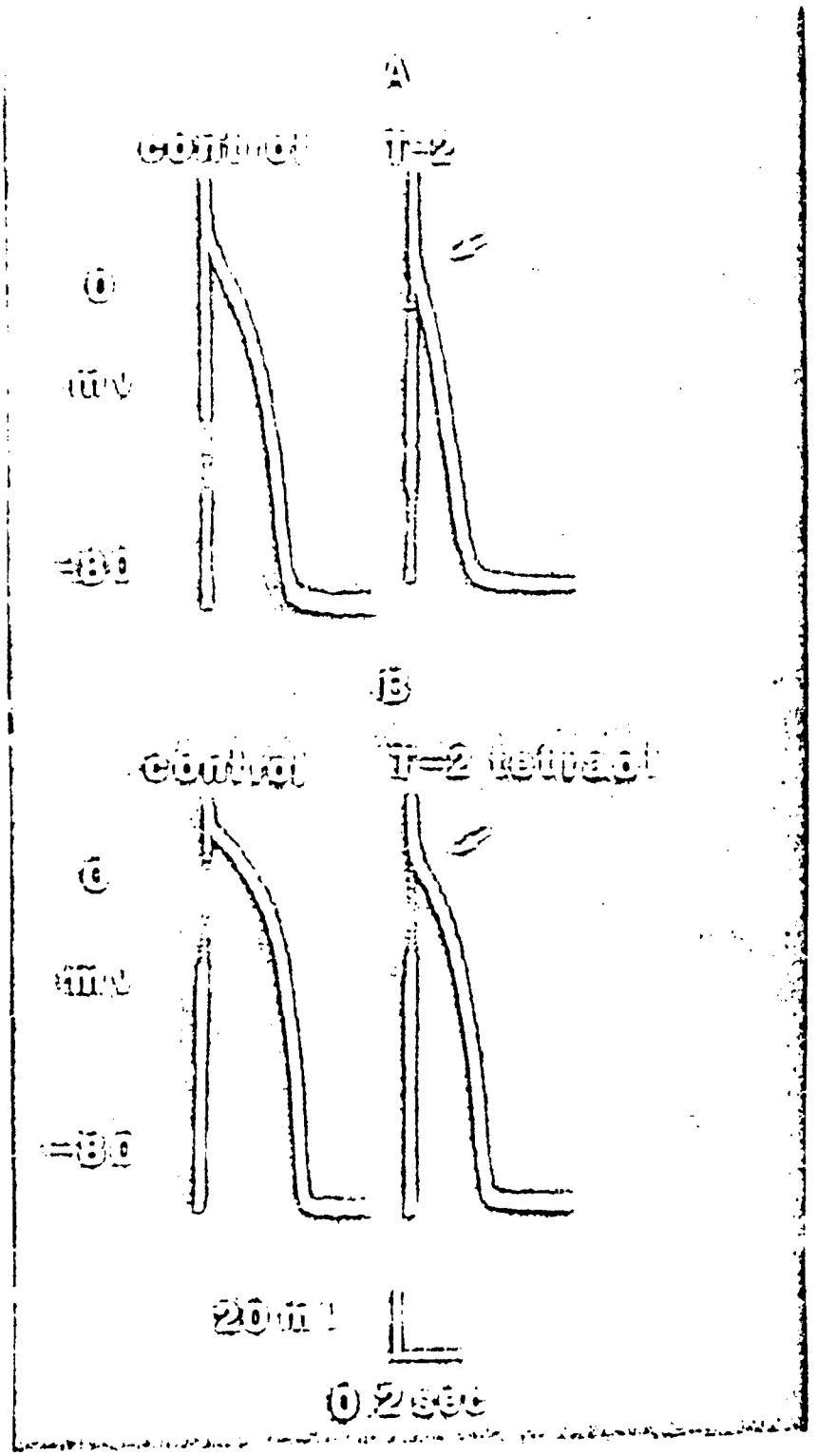

Figure 4. Action potentials from canine false tendon cells (A), and canine papillary muscle cells (B) before and after 60 minutes exposure to trichothecene mycotoxins (1 part per million).

Table 3. The effect of T-2 toxin on the action potential parameters of 3 ventricular cell types.

	AMPLITUDE (mv)	OVERSHOOT (mv)	dV/dTmax. (v/s)	COND. VEL. (M/s)	MDP (mv)	APD (20) (ms)	APD (50) (ms)	APD (80) (ms)
FALSE TENDON								
$\bar{X}$	98.3	32.1	214.3	0.538	74.4	27.9	82.9	136.4
± sd	14.6	7.2	99.3	0.178	6,7	7.6	9.9	22.1
(n)	(7)	(7)	(7)	(4)	(7)	(7)	(7)	(7)
sig.	*	ns	ns	*	*	**	**	***
PAPILLARY MUSCLE								
$\bar{X}$	87.9	17.0	139.3	0.219	79.6	49.9	111.4	134.3
± sd	7.9	4.4	19.2	0.055	5.0	16.1	11.4	9.3
(n)	(7)	(7)	(7)	(4)	(7)	(7)	(7)	(7)
sig	*	**	ns	ns	ns	***	***	***
VENTRICULAR MUSCLE								
$\bar{X}$	91.7	19.3	123.3	0.095	75.8	117.5	192.5	225.0
± sd	6.1	3.9	29.3	0.004	3.1	30.3	27.2	23.5
(n)	(6)	(6)	(6)	(4)	(6)	(6)	(6)	(6)
sig	ns	ns	ns	ns	ns	ns	ns	ns

* = $p < 0.05$, ns = $p < 0.05$ comparisons are with the control for each tissue. (see Table 1)
, MDP = maximum diastolic potential, dV/dT max. = maximum rate of rise of the upstroke, APD = action potential duration

* = $p <$ 0.01

* = $p <$ 0.001

Table 3 summarizes the effects of scirpentriol on the action potentials of canine false tendon cells, papillary muscle cells, and ventricular muscle cells. The R2, R3 hydroxylated metabolite had no significant effect on the action potential parameters of canine false tendon cells or papillary muscle cells. Ventricular muscle cell action potentials however were significantly altered by scirpentriol. The action potential duration was shortened ($p < 0.05$), the cells were depolarized by 11.5 mv ($p < 0.05$), and the total amplitude was reduced by approximately the same amount ($p < 0.05$). T-2 altered these parameters in the false tendon cell action potentials, and had no effect on the ventricular muscle cells. Scirpentriol had no effect on the false tendon cells but significantly altered ventricular muscle cell action potentials.

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Table 3. The effect of scirpentriol on the action potential parameters of 3 ventricular cell types

	AMPLITUDE (mv)	OVERSHOOT (mv)	dV/dTmax. (V/S)	COND. VEL. (M/S)	MDP (mv)	APD (20) (ms)	APD (50) (ms)	APD (80 (ms)
FALSE TENDON								
$\bar{X}$	120.7	32.0	230.0	1.120	88.7	40.0	115.0	171.7
± sd	3.1	2.0	17.3	0.453	2.3	0.0	0.0	7.6
(n)	(3)	(3)	(3)	(2)	(3)	(3)	(3)	(3)
sig	ns	ns	ns	ns	ns	ns	ns	ns
PAPILLARY MUSCLE								
$\bar{X}$	97.5	26.5	115.0	----	70.5	121.2	222.5	263.8
± sd	6.0	3.0	13.2	----	6.8	23.6	17.1	16.0
(n)	(4)	(4)	(4)	----	(4)	(4)	(4)	(4)
sig	ns	*	ns	----	ns	ns	ns	ns
VENTRICULAR MUSCLE								
$\bar{X}$	82.3	19.4	160.0	----	66.0	75.0	151.7	203.3
± sd	28.6	8.0	20.0	----	18.4	37.2	55.7	44.5
(n)	(6)	(6)	(3)	----	(6)	(6)	(6)	(6)
sig	*	ns	ns	----	*	*	**	**

* = $p < 0.05$, ** = $p<0.01$, *** = $p<0.001$. Comparisons are with the control for each tissue (see Table 1)

MDP maximum diastolic potential, dV/dT max. maximum rate of rise of the upstroke, APD action potential duration.

Table 4. The effects of T-2 tetraol on the action potential parameters of 3 ventricular cell types

	AMPLITUDE (mv)	OVERSHOOT (mv)	dV/dTmax. (V/S)	COND. VEL. (M/S)	MDP (mv)	APD (20) (ms)	APD (50) (ms)	APD (80) (ms)
FALSE TENDON								
X	123.7	28.3	291.7	0.533	95.3	40.0	191.6	251.7
±sd	12.7	3.5	12.6	0.412	12.9	0.0	46.5	37.5
(n)	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
sig.	ns	ns	ns	ns	ns	ns	ns	ns
PAPILLARY MUSCLE								
X	78.0	19.0	75.0	---	59.0	125.0	230.0	270.0
±sd	0.0	4.2	21.2	---	2.8	21.2	42.4	42.4
(n)	(2)	(2)	(2)	---	(2)	(2)	(2)	(2)
sig.	*	ns	*	---	*	ns	ns	ns
VENTRICULAR MUSCLE								
X	92.8	21.3	125.0	0.171	71.5	51.3	137.5	180.0
±sd	20.6	10.4	57.4	0.023	11.1	16.5	29.0	23.1
(n)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
sig.	ns	ns	ns	ns	ns	***	***	***

***= p 0.001, MDP = maximum diastolic potential, APD = action potential duration
** = p 0.01
* = p 0.05

Table 5.

	PAPILLARY MUSCLE (APD 50)					VENTRICULAR MUSCLE (APD 50)				
	CONTROL	ATP	T-2	T-2 + ADENOSINE	T-2 +ATP	CONTROL	ATP	SCIRPENTRIOL	SCIRPENTRIOL + ATI	
X	222.0	229.0	178.0	165.0	231.0	206.7	223.0	101.7	196.7	
±sd	16.8	17.8	8.3	7.1	29.2	11.6	17.2	16.1	16.1	
(n)	(5)	(5)	(5)	(4)	(3)	(5)	(3)	(3)	(3)	
sig.	---	ns	***	***	ns	---	ns	***	ns	

*** = p 0.001, APD 50 = action potential duration at 50% repolarization.

Plans

1. Repeat this study of ventricular cell action potentials in the specialized conduction system of the right atrium to elucidate mechanisms for arrhythmias.
2. Determine what factors (such as ATP, propranolol, veraparmil, etc.) reverse the arrhythmogenic effects of T-2, roridin A, and other trichothecenes.

ELECTROPHYSIOLOGIC ABNORMALITIES PRODUCED BY TRICHOTHECENES IN ISOLATED HEARTS (Woods and Bubien)

Table 6 shows the significant changes in isolated atrial activity that took place after 20 min. of perfusion of 4 μmolar T-2 toxin. Sinus rate fell from 222 to 142 beats per min. Action potential duration at 90% repolarization decreased from 55 to 21 msec. And the interval between activation of right atria and right ventricles increased from 48 to 70 msec. After 30 min. perfusion (or with higher toxin concentrations) disturbances in rhythm and conduction were observed.

Each Polaroid print in Figure 5 contains right atrial action potentials above and right ventricular electrograms below. The control record is Panel A. After 20 min. of 4 μmoles/L toxin perfusion, sinus rate was slower and transient periods of ventricular tachycardia were observed (Panel B). Panel C shows that whenever atrioventricular conduction did occur, the A-V interval was prolonged. Panel D shows the record after 30 min. of toxin perfusion. Atrial and ventricular tachycardia were present as was complete A-V block.

To further confirm this atrioventricular dissociation, a right atrial and a right ventricular cell were simultaneously impaled; there was no correspondence between atrial and ventricular action potentials.

Changes in sinus rate, atrioventricular conduction, and action potential morphology observed in this study can be caused by release of endogenous acetylcholine. To test this possibility, atropine (5 mg./L) was added to the perfusate to block the acetylcholine receptor. After such treatment and exposure to T-2 toxin for 30 min., there was no slowing of sinus rate and no shortening of the action potential.

Figure 6 shows the response to 30 min. perfusion of a 10X higher concentration of T-2 toxin for 20 min. The upper print shows a slow atrial firing rate, A-V block, and ventricular quiescence. 10 min. later the lower print shows long periods of atrial quiescence interrupted by brief periods of atrial tachycardia.

Table 6.

Changes in Rat Atrial Action Potentials*
after 20 Minutes of Toxin Perfusion (n = 6)

		Sinus Rate (bpm)	Action Potential Duration at 90% Repolarization (msec)	A-V Interval (msec)
Control	$\bar{x}$	222	55	48
	sd	60	3	2
Trichothecenes	$\bar{x}$	142	21	70
	sd	50	4	15

*P is less than 0.05. For resting potential, upstroke velocity, and amplitude, no significant differences were observed.

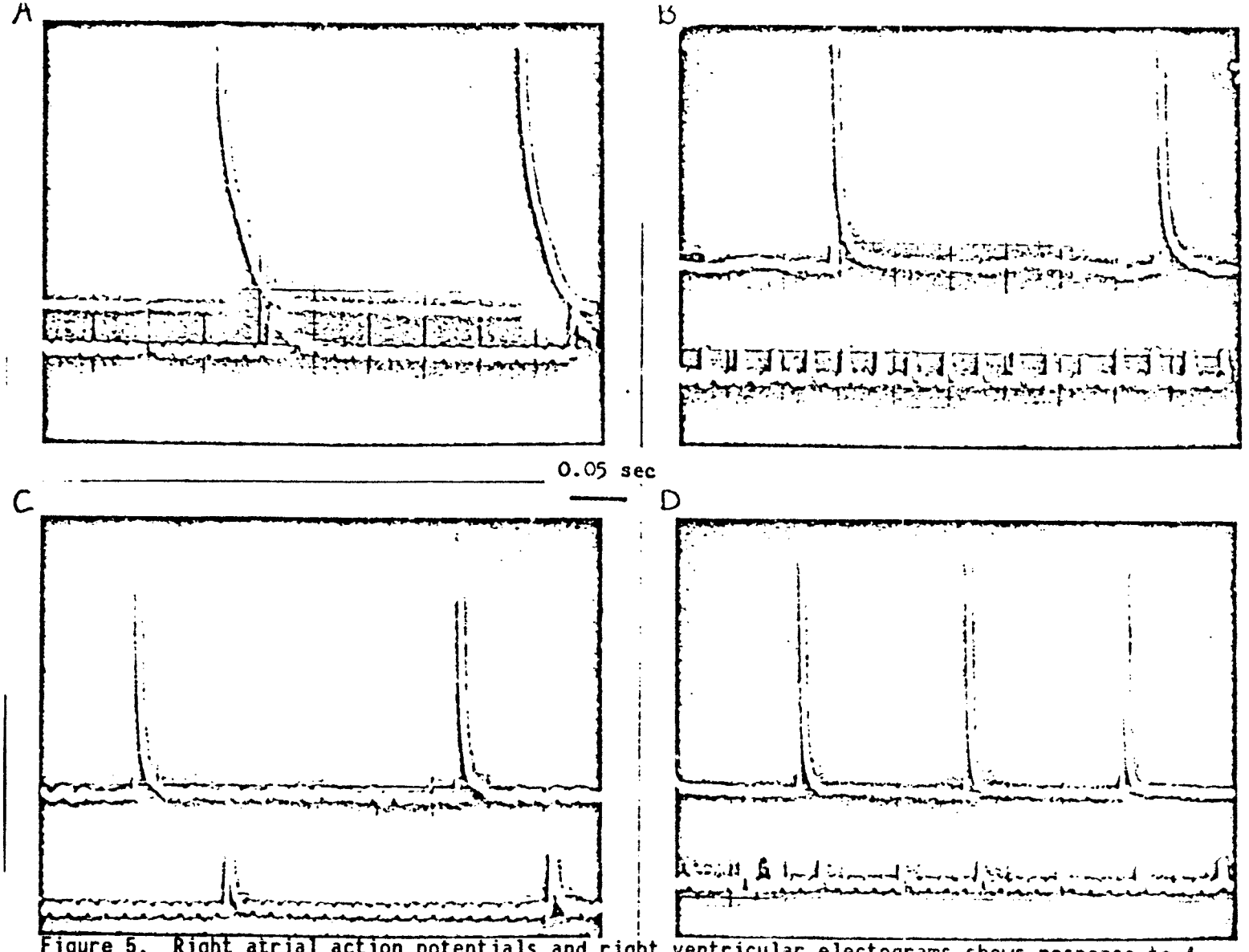

Figure 5. Right atrial action potentials and right ventricular electograms shows response to 4 micromolar T-2 toxin at 20 min (B and C) and 30 min. (D). Details discussed in text.

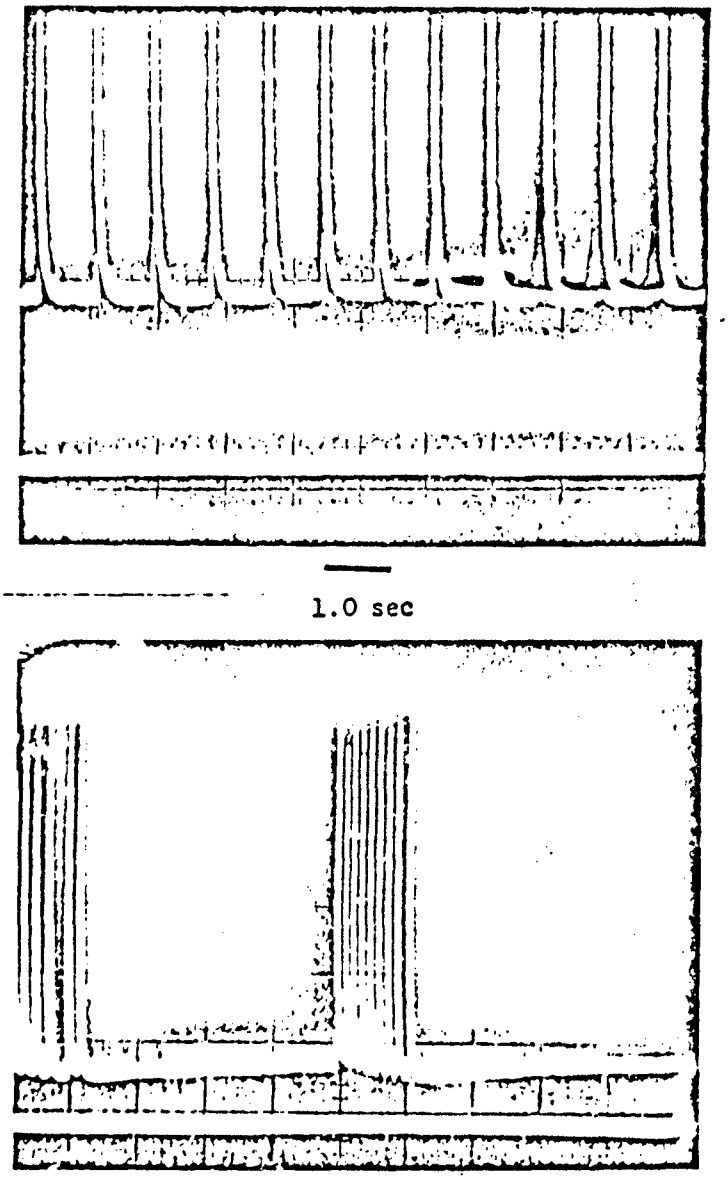

Figure 6. Recordings identical to those in Figure 5. Details are discussed in text.

In summary,

1. All trichothecenes tested up to 1 ppm or 40 µmoles/L caused atrial, ventricular, and A-V conduction disturbances.
2. Automaticity and A-V conduction were extremely sensitive to the trichothecenes.
3. Some changes were prevented by atropine, but not A-V block.
4. Effects could be reversed quickly by washout with toxin-free solution.

Plans

1. Determine whether T-2, roridin A, or other trichothecenes are selective for the cardiac cell slow channel.
2. Determine selectivity of trichothecene effects on automaticity and slow conduction versus effects on rapid conduction (through atrial and ventricular muscle cells).
3. Evaluate potential antagonists of these effects.

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